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Safety Testing and Regulation of Genetically Engineered Foods

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Abbreviations: BRAD: US EPA Biopesticides Registration Action Document, EA: Environmental Assessment, EC: European Commission, EPA: US Environmental Protection Agency, FAO: U.N. Food and Agriculture Organization, FDA: US Food and Drug Administration, FFDA: Federal Food, Drug and Cosmetic Act, FIFRA: Federal Insecticide, Fungicide and Rodenticide Act, GE: genetically engineered, MRID: Master Record Identification Number, OSTP: Office of Science and Technology Policy, PIP: Plant-Incorporated Protectants, SAP: EPA Scientific Advisory Panel, USDA: US Department of Agriculture, WHO: World Health Organization

Introduction

The use of recombinant DNA techniques to engineer food crops with novel traits has aroused tremendous interest and concern throughout the world. Both the public and the scientific community are deeply divided on a host of issues raised by genetically engineered (GE) crops. Do they pose human health or environmental risks? Are they adequately regulated? Should foods containing them be labeled? Should society allow them to be patented? Are they relevant to the developing world? Science alone cannot and will not decide the many disputes that have arisen between and within nations over GE foods. As with the introduction of any powerful new technology, economic, cultural and ethical factors will also come into play. But science can help ground the debate, particularly in the contentious area of regulation.

A thorough understanding of how GE foods are currently regulated is essential because claims regarding the safety of these crops are based largely on assessments by government regulators, which in turn are founded mostly on unpublished studies conducted by the crop developer. Published, peer-reviewed studies, particularly in the area of potential human health impacts, are rare. For instance, the EPA's human health assessment of *Bt* crops cites 22 unpublished corporate studies, with initially only one ancillary literature citation (EPA BRAD, 2001b, pp. IIB32-IIB35).¹ The paucity of peer-reviewed literature is probably due to the reluctance of companies to publish data on their crops on account of intellectual property concerns. This supposition is strengthened by reports concerning

independent researchers who have been denied GE crop material by companies, or whose access to such material is strictly conditioned (Dalton 2002). Thus, the validity of a claim that GE crop X is safe depends almost exclusively upon the quality of both the relevant corporate science and the regulatory approval process.²

Here, we will undertake a science-based critique of corporate scientific practices and the US regulatory system with respect to GE foods, with special reference to several commercialized crops and relevant (international) standards. We focus on the US regulatory system because the US has far more GE crops on the market than any other nation, and because American regulatory agencies are so often cited in support of the safety of these foods. We then outline an initial screening regimen for GE foods that, if made mandatory, would in our opinion better protect public health than the current US system.

It should be noted at the outset that this study relies heavily on material largely unknown to the broader scientific community, including several unpublished corporate studies, reports on specific GE crops and their regulation by expert bodies (e.g. committees of the National Academy of Sciences) and documents issued by US regulatory agencies. All of these sources are cited in the reference list, with web addresses where available. The general public may view and copy unpublished studies for non-commercial use at the EPA (see References). The information in this paper that derives from unpublished studies has been made available to the public previously in Freese (2001, 2002, 2003) and in presentations at forums sponsored by the FDA (Food Biotechnology Subcommittee meeting, 8/14/02) and National Academy of Sciences (Committee on Identifying and Assessing Unintended Effects of Genetically Engineered Foods on Human Health, 1/7/03).

Development of US policy

The foundation of the US regulatory system for genetically engineered foods was laid from the mid 1980s to the early 1990s during the Reagan and Bush administrations. The Office of Science and Technology Policy (OSTP 1986) and the Council on Competitiveness (Council, 1991), both White House agencies, decided early on that GE crops and foods would be regulated under existing statutes designed for invasive plants, chemical pesticides and food additives, and that use of recombinant DNA techniques *per se* would not trigger any special regulatory consideration. These policy directives led to the doctrine that later became known as ‘substantial equivalence’ (for more, see below under Food and Drug Administration). Biotech industry and government officials have testified to the great influence exerted by industry on the formulation of this policy, which was designed to speed transgenic crops to market, while at the same time reassuring consumers that GE foods have passed government review. According to Henry Miller, in charge of biotechnology at the FDA from 1979-1994: “In this area, the US government agencies have done exactly what big agribusiness has asked them to do and told them to do” (as quoted in Eichenwald *et al.*, 2001).

Regulatory purview and performance

Regulation of genetically engineered foods is divided among three federal agencies. The breakdown of regulatory responsibility is as follows:

- * The *US Department of Agriculture* oversees GE crop field trials and is responsible for deregulating (i.e. permitting the unregulated cultivation and sale of) GE crops.
- * The *Environmental Protection Agency* has jurisdiction over the pesticides in GE pesticidal plants, and has joint responsibility with the Food and Drug Administration for selectable marker genes and proteins used in crop development; and
- * The *Food and Drug Administration* conducts voluntary consultations on other aspects of GE foods with those companies that choose to consult with it.

US DEPARTMENT OF AGRICULTURE (USDA)

As of this writing, nearly 40,000 field trials of GE crops have been authorized by the USDA. 84% overall, and 98% in 2002, have taken place under a streamlined “notification” system introduced in 1993 (Caplan 2003). Under this system, the crop developer fills out an application, specifying the plant, the gene transfer method, the transformation vector, the sources of the foreign genetic sequences, and the size and location of the field trial. USDA then notifies the pertinent state department of agriculture and normally issues an “acknowledgement” within 30 days. A somewhat more involved permitting process is reserved for experimental trials involving crops engineered to produce pharmaceuticals or industrial compounds (NAS 2002).

The USDA has established guidelines (performance standards) for GE crop trials (USDA Performance 2001). The Department’s chief concern is to minimize gene flow to, and inadvertent mixing with, conventional crops and weeds. However, USDA’s recent admission that there have been 115 compliance infractions by GE crop field trial operators raises serious doubts as to the efficacy of its regulation (USDA Compliance 2003). Two contamination episodes involving field trials of biopharmaceutical corn in the fall of 2002 highlight the inadequacy of USDA’s oversight in this regard (Ferber 2003). It remains to be seen whether the Department’s subsequent strengthening of permit conditions and oversight for pharmaceutical and industrial crops will prevent contamination of food-grade crops (USDA Notice, 2003). The issue of contamination is especially important given the *de facto* zero tolerance standard for such compounds in food and feed. In addition, many of the field trial sites falling under the notification system are never visited by a USDA inspector (NAS, 2002).

USDA also clears GE crops for commercial cultivation through issuance of a “determination of nonregulated status.” As of this writing, 60 petitions for nonregulated status have been approved. Though some petitions have been withdrawn, the USDA has not explicitly denied any petitions, though one is listed as “void” (USDA Deregulated, 2003). The Department requires considerably more data for deregulation than for field trials, but deregulation is absolute, completely removing the crop and all its progeny from the USDA’s regulatory authority (NAS, 2002). In line with its governing statute, the Plant Pest Act, the USDA’s chief criterion for deregulation is the lack of invasive or “weedy” characteristics. The USDA has no authority to evaluate the potential health impacts of the crop, or of conventional crops that become contaminated with experimental traits. And since there is no mandatory review by the FDA (see below), GE crops can theoretically enter the marketplace with no review of potential health impacts.

However, even the adequacy of USDA’s evaluation of the weediness potential of a GE crop is open to question. For instance, in 1998 the USDA cleared AgrEvo’s [now Bayer CropScience] Liberty Link glufosinate-tolerant rice for commercial cultivation despite its recognition that “the *bar* gene conferring tolerance to glufosinate will introgress into red rice and could result in a glufosinate-tolerant red rice population” (USDA Determination, 1998). The USDA had earlier recognized that red rice is a weed that “causes problems in rice fields because it is carried with cultivated rice and can significantly lower its value by reducing [sic] its processing characteristics” (USDA EA, 1996). Nevertheless, the Department stated that “these hybrid offspring [glufosinate-tolerant red rice] will still be sensitive to other registered herbicides” (USDA Determination, 1998). This lack of concern is surprising in view of the USDA’s admission, in the very same deregulation notice, that varieties of rice resistant to two other herbicides (imidazolinone and glyphosate) are under development. If the USDA deregulates the latter two varieties as well, they may help foster the development of doubly- or triply-resistant weedy red rice. Multiple herbicide resistance is not unprecedented. For example, three types of canola, two genetically engineered and one mutated for resistance to a different herbicide each, are planted in western Canada. The emergence of volunteer canola plants resistant to one, two and even three herbicides is considered to be “a major weed problem” in some parts of Canada, with the potential to become “one of Canada’s most serious weed problems...” (RS Canada 2001).

A committee of the National Academy of Sciences recently reviewed the USDA's performance at regulating GE crops. Some of the many deficiencies it found include lack of transparency, too little external scientific and public review of decision-making, poorly trained personnel, and allowing companies to make excessive claims of confidential business information (CBI). In fact, the committee itself complained that it was denied access to information it needed to conduct its review due to inaccessible CBI (NAS, 2002).

ENVIRONMENTAL PROTECTION AGENCY (EPA)

The EPA's primary role is regulation of the plant pesticides in crops such as genetically engineered *Bt* corn, cotton and potatoes³. *Bt* crops are engineered to produce an insecticidal protein derived from the bacterium *Bacillus thuringiensis*. In 2003, *Bt* corn varieties comprised 29% of all US corn, while 41% of US cotton contained a *Bt* trait (NASS, 2003). *Bt* potato plantings shrank from a peak of about 50,000 acres in 1998 and 1999 to 5,000 acres in 2000, due primarily to the decision of fast-food giants McDonald's and Burger King to source only non-*Bt* potatoes (EPA BRAD, 2001d, pp. 124-125; Kilman, 2000).

The EPA is responsible not only for the environmental, but also the potential human health impacts of plant-generated GE pesticides. The EPA registers plant pesticides under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), while it has the power to set maximum allowable levels (tolerances) of plant pesticides in crops under the Federal Food, Drug and Cosmetic Act (FFDCA). The EPA has exempted *Bt* plant pesticides from tolerances in all crops (i.e. allowed unlimited amounts), save for StarLink corn, which was never approved for food use. In line with its ruling statutes, which were formulated for chemicals rather than living organisms, the EPA explicitly disavows authority over any aspects of the GE plant beyond its incorporated pesticide. This includes any potential unintended effects, which are supposedly regulated by the FDA (EPA PIP, 2001).

Unlike the FDA, which has a voluntary consultation process, companies developing GE pesticide plants must consult with the EPA. However, the EPA has failed to establish data requirements specific to plant pesticides (EPA PIP, 2001). In the meantime, the Agency has referred developers of GE pesticide-producing crops to a nearly decade-old guidance (EPA Statement of Policy 1994). This Statement of Policy devotes just 4 short paragraphs to testing for human health effects. The Agency recommends only that companies conduct short-term oral toxicity tests in rodents and *in vitro* digestibility tests on the plant pesticide, without any guidance on or specification of test conditions. One strength of EPA regulation is the Agency's ample use of Scientific Advisory Panels, outside experts called in to advise the EPA on issues where it lacks adequate expertise. However, the EPA frequently does not follow the recommendations of its expert advisers with respect to data requirements for product characterization, evaluation of potential human health impacts and specification of test conditions (see Case study - *Bt* corn below).

The quality of corporate environmental studies, and the EPA's review of them, can also be questionable. For example, feeding studies designed to detect potential effects of GE pesticidal proteins on non-target insects such as honeybees are often too short to give meaningful results, for instance 9 days (see Maggi and Sims 1994, Hilbeck and Meier 2002). However, the EPA often accepts such inadequate studies as substantiating the hypothesis that GE pesticidal proteins are not harmful to insects at the tested doses (EPA BRAD 2001a; Mendelsohn *et al.*, 2003). Hilbeck and Meier (2002) recommend full life-cycle testing to detect sub-lethal and long-term effects.

Finally, the EPA plays a critical role in the introduction of herbicide-tolerant plants by raising or establishing tolerance levels for herbicide residues on crops. For instance, in 1992 Monsanto successfully petitioned the EPA to raise the tolerance for glyphosate residues on soybeans from 6 to 20 ppm (EPA Rule, 1992). This anticipated the introduction, several years later, of glyphosate-tolerant soybeans (Lappe and Bailey, 1998), which are associated with greater usage of glyphosate

than conventional soybeans (Benbrook, 2001, 2003). The EPA recently granted a petition from Bayer CropScience, whose glufosinate-tolerant rice had already been deregulated by the USDA, to establish a tolerance for residues of glufosinate on rice (EPA, 2003).

FOOD AND DRUG ADMINISTRATION (FDA)

The US regulatory agency most commonly cited as vouching for the safety of GE foods exercises the least authority in regulating them. Theoretically, transgenic proteins in foods fall under the “food additives” provisions of the Federal Food, Drug and Cosmetic Act (FFDCA). Food additives must undergo extensive pre-market safety testing, including long-term animal studies, unless they are deemed to be “generally recognized as safe” (GRAS). The FDA has left it up to the biotech industry to decide whether or not a transgenic protein is GRAS, and so exempt from testing (FDA Policy, 1992). The FDA has yet to revoke an industry GRAS determination and require food additive testing of any transgenic crop⁴.

This blanket GRAS exemption is based on the notion of “substantial equivalence” – the strong, *a priori* presumption that GE crops are largely the same as their conventional counterparts. This assumes not only the safety of the transgenic protein, but also the absence of any potentially harmful, unintended effects of transformation. When this policy was being formulated in the early 1990s, scientists at the FDA raised numerous objections to a working draft of the policy (FDA Memos). For instance, FDA scientists at the Division of Food Chemistry and Technology and the Division of Contaminants Chemistry called for mandatory review, stating that “every transformant should be evaluated before it enters the marketplace” (FDA Memo 1991). Dr. Samuel Shibko, Director of the Division of Toxicological Review and Evaluation, recommended “a limited traditional toxicological study with the edible part of the plant,” as well as “limited studies in humans” and *in vitro* genotoxicity tests (FDA Memo, 1992a). The most commonly expressed concern was unintended effects associated with the random nature of transformation techniques. Dr. Louis J. Pribyl’s comments are typical: “When the introduction of genes into plant’s genome randomly occurs, as is the case with the current technology (but not traditional breeding), it seems apparent that many pleiotropic effects will occur. Many of these effects might not be seen by the breeder because of the more or less similar growing conditions in the limited trials that are performed.” Pribyl also raised concerns about “new, powerful regulatory elements being randomly inserted into the genome” that could cause “cryptic pathway activation” that breeders might miss. “This situation is different than that experienced by traditional breeding techniques [sic]” (FDA Memo, 1992b). Administrative superiors at the FDA and the White House apparently did not heed these concerns, resulting in today’s voluntary consultation process.

Under voluntary consultation, the GE crop developer is encouraged, but not required, to consult with the FDA. The company submits data summaries of research it has conducted, but not the full studies. That is, the FDA never sees the methodological details, but rather only limited data and the conclusions the company has drawn from its own research. As one might expect with a voluntary process, the FDA does not require the submission of data. And in fact, companies have failed to comply with FDA requests for data beyond that which they submitted initially (Gurian-Sherman, 2003). Without test protocols or other important data, the FDA is unable to identify unintentional mistakes, errors in data interpretation or intentional deception, making it impossible to conduct a thorough and critical review.

The review process outlined above makes it clear that, contrary to popular belief, the FDA has not formally approved a single GE crop as safe for human consumption. Instead, at the end of the consultation, the FDA merely issues a short note summarizing the review process and a letter that conveys the crop developer’s assurances that the GE crop is substantially equivalent to its conventional counterpart. The FDA’s letter to Monsanto regarding its MON810 *Bt* corn is typical:

“Based on the safety and nutritional assessment you have conducted, it is our understanding that Monsanto has concluded that corn products derived from this new variety are not materially different in composition, safety, and other relevant parameters from corn currently on the market, and that the genetically modified corn does not raise issues that would require premarket review or approval by FDA. ... as you are aware, it is Monsanto’s responsibility to ensure that foods marketed by the firm are safe, wholesome and in compliance with all applicable legal and regulatory requirements” (FDA Letter, 1996).

In its official capacity, the FDA carefully avoids vouching for the safety of GE foods, consistent with its voluntary review process. Clearly, the FDA does not send such letters to drug companies or makers of food additives. In these cases, the agency conducts an exhaustive review of a full set of required studies on the product, then either approves or rejects it on its own authority.

Under the voluntary consultation system, the FDA cannot adequately fulfill its role of reviewing GE foods for the presence of toxins or allergens, alterations in nutritional content, unintended effects of the transformation process, or any other food safety concerns not related to GE pesticidal proteins (which come under EPA’s purview). For example, in its consultation with Aventis on the company’s GE male-sterile corn, the FDA apparently raised no concerns about Aventis’ failure to test for possible expression of the pollen-sterilizing GE toxin barnase (a ribonuclease derived from *Bacillus amyloliquefaciens*) in kernels, leaves or other non-pollen corn tissues (FDA Note, 2000), despite evidence that bacterial barnase causes kidney damage in rats (Ilinskaya and Vamvakas, 1997; for an analysis, see Freese, 2003). Another example of the FDA’s inadequate performance is detailed below in the case study of *Bt* corn. This case study is preceded by a summary of what we believe to be the major shortfalls in voluntary corporate testing procedures.

Corporate testing procedures

Though not required to do so by the FDA, GE crop developers do test their novel plants in a variety of ways. Given the weaknesses in the regulatory system described above, the quality and scope of corporate testing become key factors in evaluating claims concerning the safety of GE crops. Three especially troubling issues are detailed below.

SURROGATE PROTEINS

Biotechnology companies rarely test the transgenic protein actually produced in their engineered crops. Instead, for testing purposes they make use of a bacterially generated surrogate protein that may differ in important respects from the plant-produced one. The same genetic construct used to transform the plant is expressed in bacteria (usually *E. coli*), and the surrogate transgenic protein is then extracted from the bacteria. This surrogate protein is then employed for all subsequent testing, such as short-term animal feeding studies and allergenicity assessments. This is, however, a serious mistake in testing paradigms, since plants and bacteria are very likely to produce different proteins even when transformed with the same gene (for discussion, see Schubert, 2002). Testing a bacterial surrogate should not substitute for testing the plant-expressed proteins for the following reasons:

DNA transfected into both plants and animals is incorporated randomly into chromosomal DNA and in doing so may disrupt the function of the chromosomal gene into which it is incorporated, contributing to the unpredictable nature of GE organisms. In addition, only part of the transfected DNA sequence may be incorporated and expressed, and additional problems arise if a fusion protein is made from both transfected and host DNA. For instance, Monsanto and Novartis developed a glyphosate-tolerant sugar beet line in which only 69% of one of the transgenes was incorporated, resulting in fusion with sugar beet DNA and production of the corresponding novel fusion protein (FDA Note, 1998). Even if precisely the same foreign DNA is expressed in bacteria and plant, the two organisms – which are kingdoms apart in biological terms – process proteins differently. For

instance, bacteria are not known to add sugar molecules to proteins, while plants do. Glycosylation patterns influence the immune response to proteins, and glycosylation is considered to be a characteristic of allergenic proteins (SAP MT, 2000, p. 23). Other secondary modifications will certainly occur when proteins are expressed in foreign organisms or different cell types (Schubert, 2002). As a result, animal feeding studies and allergenicity assessments that make use of bacterial surrogate proteins or their derivatives may not reflect the toxicity or allergenicity of the plant-produced transgenic protein to which people are actually exposed.

Biotech companies use surrogate proteins for testing purposes because they find it difficult to extract sufficient quantities of the transgenic proteins from their plants (for *Bt* crops, see: EPA BRAD, 2001b, pp. IIA3-IIA4; for glyphosate-tolerant soybeans, see Harrison *et al.*, 1996). Yet several expert bodies on both sides of the Atlantic have criticized this practice. The Scientific Steering Committee of the European Commission calls for demonstration of “chemical identity (including conformational identity)” between surrogate and plant-produced proteins before accepting the former for testing purposes (EC, 2000). According to a National Academy of Sciences committee that conducted an exhaustive review of *Bt* crops (NAS, 2000): “Tests should preferably be conducted with the protein as produced in the plant.” If surrogates are nonetheless used: “The EPA should provide clear, scientifically justifiable criteria for establishing biochemical and functional equivalency when registrants request permission to test non plant-expressed proteins in lieu of plant-expressed proteins.” Three years later, the EPA has still failed to do this, even though its scientific advisers have proposed such “test substance equivalence” criteria (SAP MT 2000, p. 14). In fact, the toxicity and allergenicity assessments of the major *Bt* corn and cotton events currently on the market employed surrogate proteins that did not meet these criteria (Freese, 2001).

Immunologic differences between plant-produced and bacterial surrogate proteins could have serious medical consequences. An EPA Scientific Advisory Panel (SAP) with some of the nation’s leading allergists was convened to evaluate cases of allergic reactions from consumption of food potentially contaminated with StarLink corn, which produces the Cry9C insecticidal protein. This SAP criticized the FDA for using a bacterial surrogate Cry9C rather than StarLink corn Cry9C in its allergy assay (an ELISA to detect antibodies to Cry9C in sera): “The use of non-equivalent, bacteria-derived coating antigen raises the possibility that IgE directed against plant derived Cry9C may not be detected.” For this and other reasons: “The test, as conducted, does not eliminate StarLink Cry9C as a potential cause of allergic symptoms” (SAP StarLink, 2001). In fact, the advisors cautioned that any level of StarLink in food might be harmful: “... the Panel concluded that based on reasonable scientific certainty, there is no identifiable maximum level of Cry9C protein that can be suggested that would not provoke an allergic response and thus would not be harmful to the public” (SAP StarLink 2001).

A protein generated in a foreign host may also exhibit point mutations relative to the native protein that can alter the protein’s immunogenicity and allergenicity (Wal, 1998). Yet regulators do not demand full sequencing data. Instead, they usually accept company studies comparing 5-25 amino acids at the N-terminal of surrogate and plant-produced proteins as sufficient for a demonstration of sequence equivalence. For example, EPA’s review of Cry1F corn states: “N-terminal sequencing of 5 aa determined that the microbial and plant expressed protein maintained this sequence intact.” Yet five amino acids represent less than 1% of the 605 amino acids in plant-expressed Cry1F (EPA BRAD, 2001c). Given the use of bacterially produced surrogate proteins as the norm for testing, one cannot avoid the conclusion that the plant-produced transgenic proteins we actually eat are virtually untested.

UNINTENDED EFFECTS

The artificial introduction of foreign genetic constructs into plant cells creates numerous opportunities for potentially hazardous, unintended effects. These include the over-production of native allergens or toxins, nutritional deficits, and, as discussed above, the creation of novel fusion proteins with

unknown properties. Unintended effects are common in all cases where GE techniques are used. For example, engineering a human gene into human cells significantly increases or decreases the expression levels of 5% of the genes in the cell (see Schubert, 2002 for discussion). Excess lignin production in *Bt* corn (Saxena and Stotzky, 2001), reduced levels of certain phytoestrogens in glyphosate-tolerant soybeans (Lappe *et al.*, 1998) and unpredicted changes in the small molecule metabolism of GE potatoes (Roessner *et al.*, 2001) are three of many examples of unintended effects in GE crops (see also Kuiper *et al.*, 2001, Haslberger, 2003).

As stated above, these issues were recognized by FDA scientists in the early 1990s, but their recommendations to require testing for unintended effects were overruled. As a result, the FDA is usually only given summary data on overall fat, protein and carbohydrate levels, together with measurements of a handful of compounds, such as amino acids and selected nutrients. In contrast, European scientists advocate non-targeted techniques for measuring the levels of hundreds of proteins, metabolites, and mRNAs to increase the chances of detecting unintended effects (Kuiper *et al.*, 2001, Kok and Kuiper, 2003), as we do below.

TEST PROTOCOLS

There are very few established protocols for assessing the potential human health impacts of GE crops. Instead, one finds loose guidelines that in most cases only list certain tests or procedures without specifying how they are to be conducted. Allergenicity test guidelines are an important case in point. Since 1996, various groups have devised so-called “decision trees” that lay out a series of tests (e.g. sequence comparison to known allergens, digestive and heat stability, sera screening, etc.) to assess the potential allergenicity of transgenic crop proteins (e.g. Metcalfe *et al.*, 1996). However, until a 2001 report by an FAO-WHO expert consultation (FAO-WHO, 2001), none of these decision-trees specified test conditions. As a result, biotech companies have been free to devise procedures of their own choosing that often vary markedly from tests conducted by independent researchers (see Case study - *Bt* corn below). Clearly, the identification and standardization of these tests is required to facilitate rigorous review. The FAO-WHO expert consultations and emerging *Codex Alimentarius* standards are a step in the right direction (Haslberger, 2003).

The following case study of *Bt* corn illustrates some of the shortcomings in corporate testing and government regulation outlined above.

Case study – *Bt* corn

Bt corn is planted on over 20 million acres in the US alone, making it the most widely planted GE crop after herbicide-resistant soybeans. Corn is a staple in many African and Latin American societies, sweet corn is popular in the US, and corn derivatives are common in processed foods. *Bt* corn therefore deserves close examination for potential human health impacts.

Bacillus thuringiensis (*Bt*) is a soil microbe that produces a variety of insecticidal endotoxins. Microbial *Bt* insecticides targeting lepidopteran pests contain *Bt* proteins of the Cry1 class, and are widely used in spray form by organic and conventional farmers to control the European corn borer (Hilbeck *et al.*, 2000). One of the major insecticidal proteins in *Bt* sprays is known as Cry1Ab. Modified versions of Cry1Ab are engineered into Monsanto’s MON810 and Syngenta’s *Bt*11 corn events. Corn hybrids descended from these two events, which were first approved by the EPA in 1996, comprise the majority of *Bt* corn in the fields. While there has been very little independent testing of *Bt* corn and other *Bt* crops for potential human health impacts, a few studies conducted on the related *Bt* sprays raise concerns about the potential allergenicity of *Bt* corn.

Our concerns derive from four sources: 1) Suggestive evidence of allergenicity from human and animal studies as well as allergen-like properties of the *Bt* insecticidal protein Cry1Ab; 2) Unintended consequences of the genetic engineering process; 3) Regulatory failure; and 4) Differences between insecticidal proteins in *Bt* sprays and *Bt* crops.

SUGGESTIVE EVIDENCE OF ALLERGENICITY

Allergic symptoms including allergic rhinitis, angioedema, dermatitis, pruritus, swelling, erythema with conjunctival injection, exacerbations of asthma, angioedema and rash have been reported in farm workers and others exposed to *Bt* spraying operations (Bernstein *et al.*, 1999). Bernstein *et al.* demonstrated that purified Cry protein extracts of *Bt* microbial pesticides containing Cry1Ab and Cry1Ac elicited positive skin tests and IgE antibody responses in two farm workers exposed to these toxins by the inhalational, dermal and possibly oral routes. Positive skin tests and the presence of IgE antibodies in serum are considered indicators of allergenicity. Though Bernstein *et al.* did not observe allergic reactions in these workers, they note that the workers were tested after only 1 to 4 months of exposure, and that “clinical symptoms would not be anticipated unless there was repeated long-term exposure...” In addition, they note that the “healthy worker effect” might have skewed their results – that is, susceptible farm workers might have associated their allergic symptoms with *Bt*, sought other employment to avoid exposure, and hence not been included in their study.

Additional evidence for the allergenicity of *Bt* endotoxins is provided by Vazquez and colleagues in a series of animal studies demonstrating that both Cry1Ac protoxin (inactive precursor of the toxin) and toxin are potent immunogens, eliciting both mucosal and systemic immune responses (Vazquez *et al.*, 1999a, 2000a), and that Cry1Ac protoxin is a systemic and mucosal adjuvant similar in potency to cholera toxin (Vazquez *et al.*, 1999b). They also found that Cry1Ac binds to surface proteins in the mouse small intestine (Vazquez *et al.*, 2000b). It should be noted that Cry1Ac is very similar in structure to the Cry1Ab insecticidal protein in most varieties of *Bt* corn. However, binding tests on Cry1Ab have yielded negative or ambiguous results. No specific binding to GI tract tissues was found in an *in vivo* test with an *E. coli*-generated surrogate Cry1Ab in rats, though some binding, described as “aspecific,” was found *in vitro* in caecum and colon tissue of the rhesus monkey (Noteborn *et al.*, 1995).

In an assessment of *Bt* crops, expert advisors to the EPA who reviewed the Bernstein study and one of Vazquez *et al.*'s four studies concluded that: “These two studies suggest that *Bt* proteins could act as antigenic and allergenic sources” (SAP *Bt*, 2000, p. 76). Different approaches were called for to further characterize the allergenic risk of *Bt* proteins: “Only surveillance and clinical assessment of exposed individuals will confirm the allergenicity of *Bt* products...” (SAP *Bt*, 2000, p.76). Finally, the EPA's experts noted that testing for potential reactions to Cry proteins in *Bt* spray and *Bt* crops could be undertaken now: “The importance of this [Bernstein's] report is that reagents are available that could be used for reliable skin testing and serological evaluation of *Bt* protein exposed individuals.” Unfortunately, in 2001 the EPA re-registered *Bt* corn for 7 years without making use of these reagents (EPA BRAD, 2001d, p. I2). The Agency has also discounted other evidence of the potential allergenicity of *Bt* proteins.

This evidence relates to physical characteristics of the *Bt* corn protein (Cry1Ab) that are considered typical features of food allergens by expert groups that have devised decision-tree protocols designed to screen novel transgenic proteins for allergenic potential (e.g. Metcalfe *et al.*, 1996, FAO-WHO, 2001). Three of these characteristics are amino acid sequence homology to a known allergen, digestive stability and heat stability. While none of these features is *predictive* of allergenicity, their presence (especially in combination) is regarded as sufficient evidence to reject the pertinent GE crop, or at least trigger additional testing, depending on the protocol. While the EPA ostensibly “requires” data on these three parameters for all *Bt* crop proteins “to provide a reasonable certainty that no harm will result from the aggregate exposure” to them (EPA BRAD 2001b, p. IIB1), in practice it has simply not collected pertinent studies, accepted substandard ones, or ignored relevant evidence.

For instance, the EPA apparently did not make use of a study by FDA scientist Steven Gendel that demonstrated sequence homology between several Cry proteins and known food allergens. Homology of sequences 6 to 8 amino acids in length are considered potentially significant because allergenic epitopes can be this small (Metcalf *et al.*, 1996, FAO-WHO, 2001). Gendel found that Cry3A (*Bt* potatoes) and β -lactoglobulin, a milk allergen, shared sequences 7-10 amino acids in length. He also identified sequences of 9-12 amino acids shared by Cry1Ab (*Bt* corn) and vitellogenin, an egg yolk allergen. Gendel concluded that: "...the similarity between Cry1A(b) and vitellogenin might be sufficient to warrant additional evaluation" (Gendel, 1998). The EPA knew about this study because it had been discussed by its scientific advisers (SAP MT 2000). But the Agency re-registered *Bt* corn for 7 years in 2001 without discussing or even citing Gendel's study in its review document, with no corresponding study on file from Syngenta, and only incomplete data from Monsanto (EPA BRAD, 2001b, p. IIB4).

Many food allergens are stable to digestion. It is thought that the longer a protein survives in the gut, the more likely it is to induce the cascade of immune system events leading to allergic sensitization and reaction in susceptible individuals. Most food proteins, both native and transgenic, break down rapidly in the gut due to the action of protein-degrading enzymes and acid. Transgenic proteins (or rather, their bacterial surrogates) are normally tested *in vitro* in acidic solutions containing pepsin. The rate of breakdown is significantly influenced by the amount of pepsin relative to test protein in, and the acidity of, the simulated gastric fluid.

Two digestive stability studies on Cry1Ab, the GE toxin found in *Bt* corn, by Hubert Noteborn established that: 1) After 30-180 minutes in simulated gastric fluid (SGF), 9-21% of Cry1Ab remains undigested; 2) After 2 hours in SGF, Cry1Ab degrades only to fragments of substantial size at the low end of the range considered typical of food allergens (15 kilodaltons); and 3) Cry1Ab is substantially more resistant to digestion than four other transgenic proteins tested, including one other Cry protein, Cry3A. Of the six proteins Noteborn tested, only StarLink corn's Cry9C exhibited greater digestive stability (Noteborn *et al.*, 1995, Noteborn, 1998). In contrast, industry procedures used to measure digestive stability frequently employ highly acidic conditions and a very large excess of pepsin relative to test protein – conditions that favor the most rapid possible digestion (e.g. Ream 1994). Under the authoritative allergenicity testing protocol recommended by international experts at FAO/WHO, digestive stability tests are to be carried out at a higher pH (2.0) and in SGF with a ratio of test protein to pepsin over three orders of magnitude greater than the conditions used by some (FAO-WHO, 2001). Thus, it's no surprise that protein stability results may vary by a factor of up to 60. These conflicting reports show the need for standardized testing procedures.

Finally, Noteborn also found that Cry1Ab possessed "relatively significant thermostability ... comparable to that of the Lys mutant Cry9C protein" found in StarLink corn (Noteborn, 1998). Noteborn found that Cry9C was stable for 120 minutes at 90° C, but gives no further information on Cry1Ab's heat stability. The EPA failed to collect any heat stability study from Monsanto on MON810 (EPA BRAD, 2001b, p. IIB4). For further analysis of the data discussed above, see Freese (2001).

UNINTENDED CONSEQUENCES OF THE GENETIC ENGINEERING PROCESS

Many *Bt* corn hybrids planted on millions of acres in the US are derived from Monsanto's MON810 event, which contains the Cry1Ab insecticidal toxin discussed above. However, an unpublished molecular characterization study on MON810 reveals that the genetic construct broke apart during the transformation process, resulting in several unintended consequences (Levine *et al.*, 1995). The following aberrant transfection events were noted: 1) An undefined portion of the E35S enhanced cauliflower mosaic virus promoter was incorporated into MON810; 2) Only a fragment (about 70%) of the intended full-length cry1Ab protoxin gene was incorporated; 3) Thus, by definition the NOS termination sequence was not integrated; instead, the cry1Ab gene fragment fused with enough DNA

to code for 2 amino acids (Levine *et al.*, 1995), DNA that apparently derives from the host plant. These unexpected transfection events create the potential for production of a fusion protein. Yet Western blots apparently did not reveal the predicted expression product of the open reading frame, a 92 kD fusion protein, but rather only a 63 kD “tryptic core” protein. Levine *et al.* speculate that their failure to detect the putative 92 kD fusion protein is “probably due to low expression or rapid degradation to the trypsin-resistant product during the extraction procedure.” The authors do not report any formal experiment to test either of these possibilities.

In addition, Lee *et al.* (1995) and Lee and Bailey (1995) report that the safety testing for MON810 and related *Bt* corn lines employed a bacterial surrogate Cry1Ab made in *E. coli*, not the fusion protein apparently produced by MON810. These two studies attempt to demonstrate equivalence between the plant-produced and bacterial surrogate Cry1Ab proteins to justify use of the latter in safety testing, yet the equivalence testing compared only the trypsin-generated cores of the plant and bacterial proteins. Results of testing with this bacterial surrogate clearly may not reflect the toxic and allergenic profile of the putative corn-produced fusion protein. Thus, the properties of the plant-expressed protein remain largely unknown (see Freese, 2001 for a fuller discussion).

Whatever partial *Bt* fusion protein is produced by MON810, it confers insect resistance, the crop developer’s chief concern. But regulatory officials should demand more. The EPA, which has jurisdiction over the plant pesticide, merely noted in its review document that MON810 produces a “truncated” Cry1Ab protein (EPA BRAD 2001b, p. IIA6), saying nothing about integration of a gene fragment or generation of a fusion protein. The FDA, which is supposed to review the whole GE plant (even pesticidal plants like MON810) for unintended effects, nutritional deficits, etc., states in its consultation note that MON810 contains 1 complete copy of the cry1Ab gene, a NOS termination sequence, and a “nature-identical” Cry1Ab protein, none of which is correct (FDA Note 1996). Apparently, either Monsanto submitted incomplete summary data to the FDA, or the FDA made serious errors in its consultation note. In either case, it is troubling that the US agency responsible for food safety has fundamentally flawed molecular characterization data on such a widely planted GE crop. In general, we believe that the presence or potential presence of a novel fusion protein in a GE crop should trigger a mandatory review for potential human health or environmental impacts.

Bt corn exhibits another striking unintended effect. *Bt* corn hybrids descended from Monsanto’s MON810 and Syngenta’s *Bt11* events have markedly increased levels of lignin in stem tissue (Saxena and Stotzky, 2001). This finding is in accord with anecdotal reports from farmers that *Bt* corn is stiffer and less desirable to farm animals as fodder, for lignin is the woody component of plants and is non-digestible. Lignin is the polymeric product of three aromatic compounds, coniferyl alcohol, p-coumaryl alcohol and sinapyl alcohol, all of which are derived from phenylalanine, an essential aromatic amino acid (Humphreys and Chapple, 2002). Phenylalanine, in turn, is a product of the shikimic acid pathway, which is responsible for generating compounds comprising 35% and more of the dry mass of higher plants (Alibhai and Stallings, 2001). The discovery of increased lignin levels in *Bt* corn raises the question of whether other metabolic intermediates or products associated with the lignin and shikimic acid biosynthetic pathways have been affected by the transformation process. Aromatic biomolecules are extremely important in both plants and mammals as building blocks for hormones and other bioactive substances. The limited testing of these crops might easily have missed unintended increases or decreases in the levels of these other bioactive substances.

Finally, the finding that two completely different transformation events (MON810 and *Bt11*) are both associated with increased lignin levels raises an interesting question. Normally, one would expect that each non-repeatable, unique transformation event would yield unique unintended effects related to copy number, the site(s) of insertion, or other factors unique to the event. Finding the same unintended effect in two different transformation events suggests that the genetic transformation process *per se* (here, particle bombardment) might be responsible for an increase in lignin levels, and perhaps other undetected effects. Another possibility is that the cry1Ab gene or gene product exerts a lignin-promoting effect. The increased lignin content of *Bt* corn was brought to light only 5 years after market introduction. The lack of targeted testing for other bioactive substances associated with

the lignin and shikimic acid pathways, and the failure to apply non-targeted techniques such as metabolic profiling and long-term animal feeding studies, highlight the serious gaps in the human health assessment of *Bt* corn.

SIMILARITIES AND DIFFERENCES BETWEEN *BT* SPRAYS AND *BT* CROPS

The EPA's chief justification for approval of *Bt* crops in the absence of crucial data is that *Bt* sprays have a history of safe use, and so *Bt* crops are presumed to be safe as well. This presumption is not justified for several reasons. First, it is reasonably clear that *Bt* sprays do cause allergic symptoms, as detailed at the beginning of this case study. Expert advisers to the EPA told the Agency that more studies are needed to determine the allergenic risk posed by Cry proteins in general – whether from *Bt* sprays or crops (SAP *Bt*, 2000). Secondly, there is likely much greater chronic exposure to Cry proteins in *Bt* crops than in sprays. Cry proteins in *Bt* sprays break down within several days to two weeks upon exposure to UV light (Ignoffo and Garcia, 1978; Behle *et al.*, 1997), while this is obviously not the case with *Bt* crops, which produce the toxin internally in grains and other plant tissues. Thirdly, *Bt* sprays are composed primarily of endotoxins in an inactive crystalline form. They are only toxic to insects with alkaline gut conditions that permit solubilization of the crystal to the protoxin, followed by proteolytic cleavage to the active toxin (Hilbeck *et al.*, 2000). *Bt* crops, on the other hand, are generally engineered to produce the *Bt* toxin (e.g. *Bt11*), which is active without processing, or a somewhat larger fragment (e.g. MON810). There is also evidence indicating that Cry toxins are more immunoreactive than Cry protoxins (Freese, 2001). Finally, the trend to increased Cry protein expression fostered by the EPA's "high-dose" strategy to slow development of pest resistance to *Bt* crops (EPA BRAD 2001e) may result in an increase in consumers' dietary exposure to *Bt* proteins. For instance, Mycogen/Pioneer's Herculex Cry1F corn, registered in 2001, expresses at least an order of magnitude more Cry protein in kernels than MON810 (Mendelsohn *et al.*, 2003). Use of chloroplast transformation, while still at the experimental phase, raises *Bt* protein levels still higher (Kota *et al.*, 1999). Thus, even if one ignores the evidence of allergenicity and concedes that *Bt* sprays have a history of safe use, this is clearly not adequate grounds on which to judge *Bt* crops and their incorporated plant pesticides as safe.

BREAKDOWN IN THE REGULATORY SYSTEM

The question of whether *Bt* corn hybrids are harmful to consumers is still open. Testing along the lines indicated below is urgently needed to address this potential problem. However, even if no adverse effects were discovered, this case study dramatically illustrates the fundamental flaws in the US regulatory system for genetically engineered crops. Consider the following:

- (1) the EPA registered, and in 2001 reregistered, Monsanto's and Syngenta's *Bt* corn events without following up on suggestive evidence of allergenicity, in particular, studies demonstrating Cry1Ab's amino acid homology to a known food allergen and stability to digestion;
- (2) the EPA approved MON810 on the basis of studies that employed a derivative of a surrogate bacterial protein rather than the plant-produced protein;
- (3) neither the EPA nor the FDA demanded characterization of the novel *Bt* fusion protein apparently produced by MON810;
- (4) to our knowledge, there has been no published effort to investigate the potential health implications of a marked, unintended effect of the engineering process – namely, increased lignin levels in *Bt* corn stalks; and
- (5) the FDA's flawed consultation document on MON810 reveals the fundamental weakness in its review practices.

Genetically engineered crops have been on the market for a decade, are planted on 58.7 million hectares worldwide (James, 2002), and have entered the diets of hundreds of millions, mostly without their informed consent. The unique risks posed by recombinant DNA technology applied to plants

and the prevalence of foods containing ingredients derived from them demand adherence to extremely high standards of food safety. We have outlined some of the serious shortfalls in corporate testing procedures and US regulatory oversight for GE foods. Below we outline a testing regimen that we believe would better detect potentially harmful changes in GE foods and so better protect public health. While the manuscript was in preparation, a somewhat similar set of initial screening tests, in particular metabolic profiling, was proposed by Kok and Kuiper (2003).

Safety testing procedures

The previous paragraphs outline our concerns with an undefined and haphazard set of regulations and voluntary testing procedures that are applied to GE foods in the US. They show that in many cases there is no testing of the plant product that is actually consumed. Instead, a bacterially produced surrogate protein is usually used. However, it is unambiguously clear that the inserted gene, when expressed in plants, directs the expression of a protein that can be modified in a large number of ways so as to render it distinct from the version made by bacteria (Schubert 2002). The expression of a foreign gene in a plant can also dramatically alter the metabolism of the host, resulting in the production of an altered array of gene products and low molecular weight metabolites (Roessner *et al.*, 2001). Our understanding of the science makes it clear that the genetic regulatory events resulting from the random insertion into the plant chromosome of a foreign gene driven by a viral promoter are going to be distinct from those caused by moving around linked blocks of genes through recombination or even increasing their number by chromosome duplication. At present, we do not understand the mechanisms of GE-induced changes in gene expression in sufficient detail to make an outcome prediction of the type that can be made when crossing two strains such as wheat that have been eaten safely for thousands of years. Even with outcrossing to wild relatives, very few deleterious genes have been introduced into crops (Gepts, 2002). Since postmarket epidemiology is impossible in the absence of labeling, and genetic manipulations are essentially irreversible, we must get it right the first time. While US regulators, as outlined above, have made testing for potential health and environmental impacts optional and non-rigorous, the European Union, driven partly by informal public opinion, has adopted something akin to the precautionary principle. Perhaps the most extreme form of this concept was introduced by the French mathematician Blaise Pascal when he argued that even if you thought that it was very unlikely that a vengeful God existed, it was well worth your time and effort to behave as though he did, because making the extra effort for a short time to be good on earth would be much better than spending an infinity being tortured in hell. Therefore, European regulators argue that they are not prepared for the introduction of GE food until the long-term ecological and health consequences of these plants are better known, and they are willing to work a little harder to keep the public informed, for example, by requiring stringent labeling of GE products as well as the ability to trace the GE material to its origin (EC, 2003). In addition, it has been shown that the US regulatory system, based upon a weak interpretation of substantial equivalence (SE) that treats it as the end point rather than the starting point of evaluation, is substantially lacking in rigor and cannot be used to declare a product as safe as its conventional counterpart. It is therefore likely that many nations will require a more scientifically valid testing regimen than that used in the US. What should these more rigorous tests look like? While we believe that the concept of SE is valid as a starting point, it clearly cannot be demonstrated merely with gross compositional analyses showing similar levels of protein, fat, starch, and perhaps selected nutrients and antinutrients in the GE and conventional plant, as in the US system. The transfection event used to create a GE plant generates unpredictable changes in gene expression that are going to be different in kind from those produced by traditional breeding. Therefore, testing must include screens for random changes in addition to the examination of potential problems that may be predicted from the expression of the transgene itself. The following paragraphs review some published test procedures and suggest a few additional testing criteria that should be useful in predicting the potential long-term health effects of a GE food.

To a large extent, many of the proposed schemes for testing GE foods suffer from the same erroneous assumption that is made by those who develop these products. That is that the insertion of a specific

genetic sequence produces a phenotype that is related to, although perhaps somewhat divergent from, that produced by the gene in its normal host and cellular environment. While this may sometimes be the case, it is certainly not the rule, for totally unpredictable changes unrelated to the nature of the transgene can occur. This is because of the complexity of interactions between genes as well as the more obvious problems of gene disruption by insertion of the transgene itself. Unintended effects also arise with conventional breeding, but these usually occur in a limited and well-studied group of cultivars and are eliminated by backcrossing to make isogenic strains. Since GE plants may contain multiple insertion sites and chromosomal instabilities may result from the activation of dormant transposons (Meyer, 1999; Courtial *et al.*, 2001), unintended traits are not always inherited in a Mendelian manner, and productive backcrossing to yield genetically stable cultivars is difficult. Transposon activation also occurs during normal breeding, resulting in unpredictable gene insertions. This natural process, however, is very distinct from GE gene insertion. The transposed gene is not linked to a viral promoter to drive continuous expression and the GE insertion is strongly and artificially selected for in culture, while the transposon event in wild type plants is rare and subject to natural selection. Finally, sites of transposon insertion are not completely random throughout the chromosome, and may be quite distinct from the insertion sites of engineered genes. Therefore, while it is very important to determine the sequence of the inserted gene and gene product to identify possible allergenic sequences, potentially toxic fusion proteins and other novel products, it is also necessary and perhaps more efficient to use existing technology to initially do more global non-targeted screens for potential problems in three areas. These are screens for mutagens via the AMES test, for the introduction of toxic metabolic intermediates or the loss of nutrients by metabolic profiling, and for teratogenesis and other adverse effects by feeding experiments over several generations with laboratory animals. By establishing an accepted range of traits within a family of cultivars in various environments, the introduction of the GE plant could be rapidly stopped if it falls outside of the normal distribution. A fourth screen, DNA chip analysis for gene expression, gives a good overview of changes in gene expression, and may be useful for the identification of specific toxins and antigens. However, at this point it has little additional predictive value as far as safety, and is available only for species where the genomic sequence is known, such as rice.

Of the first three screens, the AMES test is a very good predictor of the mutagenicity potential of a compound (Maron and Ames, 1983), and is a complement to the FDA requirement of long-term (2-year) carcinogenicity testing in animals for drug approval. This assay makes use of the fact that a nonvirulent strain of *Salmonella typhimurium* can grow in culture medium without amino acids. Defined mutants of the bacterium have been selected that require histidine for growth. Since carcinogens will cause mutations that reverse the original mutations, the carcinogenic potential of a compound or extract can be very simply assayed by the ability of the treated cells to grow on histidine-free medium. This assay has been adapted for assaying carcinogens with different specificities and is widely used throughout the world. It has been used extensively in the field of plant biology (Elgorashi *et al.*, 2003), but has not, to our knowledge, been used for GE food safety screening. It is simple and very inexpensive. Mutagenicity screening with the AMES test and the metabolic profiling discussed below would initially require baseline determinations of perhaps six widely planted cultivars of a particular crop, such as corn, including the parent of the GE line, grown under a variety of conditions. Once this is done and a distribution of mutagenic potential and individual metabolites is determined, using the part of the plant that is eaten, then it would not be necessary to repeat these assays. It is anticipated that a distribution of mutagenicity will be found, with each data point dependent upon the cultivar and the growth conditions. The GE crop would be grown under a similar set of conditions and its mutagenicity and metabolites characterized. If it falls within the normal distribution for toxic compounds, then it should be considered as passing the criterion; if not, it should be disallowed. A less permissive standard of comparison – perhaps the non-engineered isoline control – would be more appropriate for nutrients and other beneficial compounds.

Metabolic profiling is a process that uses the modern technologies of chromatography and mass spectroscopy to identify low molecular weight molecules made by cells, many of which are involved in normal metabolic processes such as energy metabolism (Trethewey *et al.*, 1999). However, plants make additional small molecules, such as the amino acids beta-N-oxalylamino-L-alanine (BOAA)

from chickpeas and beta-methylamino-L-alanine (BMAA) from cycads, which can act as excitotoxins and cause serious neurological damage (Meldrum, 1993). It is the deregulation of the synthesis of low molecular weight toxins, mutagens and carcinogens caused by GE that has the potential to be the single greatest long-term health risk entailed by this technology. In addition, plants make a very large variety of nutrients and antioxidants whose loss or reduction could have serious adverse consequences for human health. Many of these can also be quantitated with metabolic profiling techniques (Roessner *et al.*, 2001; Schmelz *et al.*, 2003). Therefore, using a relatively small number of analytical procedures, it should be possible to quantitate many of the known nutrients, antioxidants, mutagens, carcinogens and toxins in a plant.

As alluded to above, essentially all plants naturally contain small but significant quantities of toxins, mutagens, and carcinogens (Ames *et al.*, 1990). Through many millennia of selective breeding, the levels of most of these noxious compounds have been minimized in our modern food crops. While it is not impossible to reactivate a toxin-producing pathway by normal breeding procedures (indeed, screening is always done in genera known to produce toxins), the unique technologies used to produce GE crops could activate dormant toxin pathways in species usually not associated with specific toxins. Therefore, the non-targeted approach for toxin screening should be more useful than trying to test for specific toxins based upon prior knowledge. Aside from the identification of toxins and nutrients, the majority of the information obtained from metabolic profiling may initially have no predictive value, but once large numbers of both wild type and GE cultivars are examined it may be possible to identify patterns of metabolic changes caused by GE that will produce an undesirable phenotype. For example, there are metabolic stress responses in plants as there are in animals. Many of the products of these stress-response pathways are beneficial to both plants and humans. For example, phenolic antioxidants are frequently produced by plants in response to stress. The production by grapes of the potent antioxidant resveratrol in response to mold infection both kills the invading mold and promotes longevity in some species (Howitz *et al.*, 2003). If genetically engineering a plant were to trigger loss or reduction of this group of compounds, it could be identified and quantified by metabolic profiling.

An aspect of food safety testing which in our opinion has been grossly neglected is the use of animals. The lab mouse is the work horse of FDA drug screening programs, and is used to determine the safety of a product, particularly its effects on reproduction and development. No matter how much *in vitro* data are accumulated, it is impossible to determine if a product is safe unless it is tested in an animal. The FDA has long recognized this fact, and the plant biotech companies must also. The FDA requires an extensive, but not necessarily complex, series of safety tests to be performed, largely in mice, before any drug or even a food additive can be tested in humans. A few of these assays are easily adapted to testing plant material. In our opinion, the most critical tests are those for chronic toxicity, reproductive performance, and potential teratogenic effects by long-term feeding of the GE product, using the parental non-GE material as a control. It is frequently argued that it is hard to keep an animal healthy on a test diet and that the assay is irrelevant because people simply do not eat that much of a single food. The latter is clearly not true. According to Dr. Drinah Nyirenda, director of the Program Against Malnutrition in Zambia, a typical Zambian diet, for example, is 70% corn (Daily Democrat, 2003). The former problems can be circumvented by feeding the animals a balanced diet of which the test material is the major component, but not the only one. The goals of the chronic animal tests are to determine if any organs are susceptible to toxicity, to examine overall growth rate and health, and most importantly, to determine if the GE material has any effect on litter size (fertility) and other aspects of development (teratogenicity). These studies are critical because embryogenesis is an exquisitely fine-tuned process controlled by ultra-low levels of small molecules such as steroids and retinoids. Plants can make related molecules that may interfere with normal development. Over the past 10,000 years, it is likely that plant varieties that have adverse reproductive effects have been eliminated from our food supply, but modern GE technology may accidentally activate dormant pathways that adversely affect development. Feeding the GE plant to mice for a few generations would generate some assurance that this has not occurred.

The above paragraphs outline three non-targeted safety screening procedures that have not been extensively discussed in the context of GE food. A safety issue that has received more attention is the potential for genetic engineering to introduce novel allergens into food crops. The Edmonds Institute (1998) has proposed a series of tests to screen novel proteins for potential allergenicity. As discussed above, experts at the Food and Agriculture Organization and World Health Organization have also formulated an authoritative decision-tree testing protocol that involves structural comparison of the novel protein to known allergens, various *in vitro* tests (e.g. digestive and heat stability), and screening for IgE binding with sera from allergic patients (FAO-WHO, 2001). The importance of this particular protocol is that it represents the best thinking of international experts, serves as the basis for the authoritative *Codex Alimentarius* international food safety standards, and for the first time specifies detailed test parameters. As noted above in the case study, varying test conditions have given rise to widely divergent results for parameters such as digestive stability. Though testing in animals would be desirable to supplement *in vitro* testing, this must await development of a good animal model.

Many plant allergens remain unknown or uncharacterized. Nevertheless, it is widely agreed that the predicted amino acid sequence of novel transgenic proteins should be checked for sequence homology to all known allergens. FAO-WHO (2001) recommends overall sequence comparison as well as a stepwise comparison of 6-amino acid subsequences (based on minimum epitope length), with clear specification of pass and fail criteria. Kleter and Peijnenburg (2002) recently applied FAO-WHO procedures to a group of 33 transgenic proteins in a two-step procedure designed to eliminate false positives. One transgenic protein that passed both cuts in their procedure was glyphosate oxidoreductase (GOX), a secondary mechanism for glyphosate resistance used in some varieties of glyphosate-tolerant canola and corn. It was found to have a subsequence that matched part of a proven allergenic epitope in a shrimp allergen (Kleter and Peijnenburg, 2002). Though not incorporated in the FAO-WHO protocol, Gendel (1998) argues persuasively for comparison procedures that allow for substitution of biochemically similar amino acids.

However, the remaining tests require protein, and it must be stressed that only protein produced by the part of the plant that will be eaten should be used, not a bacterially expressed surrogate protein, as is often done. Once again, FAO-WHO (2001) standards should be applied. Unlike earlier protocols, FAO-WHO specify the composition of simulated gastric fluid to be used for such tests (i.e. ratio of pepsin to test protein, pH) as well as breakdown evaluation criteria (i.e. how small must digested fragments be to qualify as “digested”). The FAO-WHO protocol also establishes procedures for testing novel proteins against IgE from individuals with known food allergies, with different sera testing procedures for GE proteins from source organisms with and without a known history of allergenicity. Nevertheless, the possibility that a previously unknown allergen can be introduced is a strong argument for labeling foods such that they can be traced to the point of origin. FAO-WHO also recommends consideration of postmarketing surveillance, in analogy to the final phase of drug testing, to capture allergic responses that may be missed with pre-market testing (FAO-WHO, 2001).

It seems to us that the safety testing procedures briefly outlined above – the Ames test for mutagenicity; metabolic profiling for toxic and nutritional compounds; extended animal feeding for carcinogenic, reproductive, teratogenic and other adverse effects; and allergenicity testing – should be sufficient, in conjunction with standard crop testing procedures, to determine if a new GE product falls within the accepted norm of safety of current food crops. All of the assays are straightforward, relatively inexpensive, and their uniform implementation would serve at least as a starting point for a rational testing regimen that may satisfy many science-based critics of this technology. Other scientific concerns stemming from the potential health risks of outcrossing and the expression of transgenes in different genetic backgrounds and growth conditions are more complex and have only recently been addressed (Haslberger, 2003). Obviously, the other ecological, political, social and economic issues surrounding genetically engineered crops are even more complex and will require a great deal more work to achieve a fair and equitable solution for all concerned.

Conclusion

In the preceding paragraphs, we have described the US regulatory system for GE foods, and with specific examples pointed out serious deficiencies in both regulatory oversight and corporate testing procedures. It is clear that the US regulatory process must be made mandatory, as well as more stringent and transparent. Any legal obstacles standing in the way of a thorough, mandatory, premarket review process must be overcome, with new statutes specifically designed for genetically engineered foods. Truly sound science must prevail in the debate over genetically engineered foods to ensure the safety of both consumers and the environment. The outline for an initial screening regimen proposed here offers an additional step toward this end.

Endnotes

¹ At the prompting of public interest groups and the Agency's scientific advisers, the EPA gave cursory treatment to four additional literature studies.

² In the US, this ill-chosen term, which seems to pre-judge the outcome of regulatory consideration, has come to replace the more neutral "review process."

³ Recently renamed "plant-incorporated protectants." The EPA's role in regulation of antibiotic and herbicide resistance marker genes/proteins will not be addressed here.

⁴ The Flavr-Savr tomato, engineered for longer shelf life, was subjected to a somewhat more stringent review only at the request of its developer, Calgene.

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(Note: Unpublished studies submitted to the EPA and identified with MRID numbers are available for inspection at the EPA at: Public Information and Records Integrity Branch (PIRIB), Room 119, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, Virginia, from 8:30 a.m. to 4 p.m., Monday through Friday, excluding legal holidays. The PIRIB telephone number is (703) 305-5805)

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